Crystal structure of Met8p, a bifunctional NAD-dependent dehydrogenase and ferrochelatase involved in siroheme synthesis.

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INTRODUCTION

Siroheme, the prosthetic group required for the six-electron reduction of both sulfite and nitrite, is a modified tetrapyrrole that is structurally related to heme, chlorophyll and cobalamin. The synthesis of all tetrapyrroles begins with the production of Uroporphyrinogen III from a five carbon precursor. In *Sacchromyces cervisiae*, siroheme is produced through four additional modifications carried out by two enzymes. Met1p catalyzes the AdoMet-dependent methylation of carbons 2 and 7 to produce precorrin-2. Siroheme is produced through an NAD-dependent oxidation of the ring to produce sirohydrochlorin followed by iron chelation (Figure 1). These last two steps are carried out by the bifunctional enzyme, Met8p [1].

The structure of Met8p has been solved to 2.1 Å resolution using SeMet MAD phasing. The structure reveals a unique, intertwined homodimer in which each monomer contains one typical NAD-binding domain, and two additional domains. While the dehydrogenase active site is delineated by the presence of bound NAD, the chelatase active site is yet unknown. Structure-based site-directed mutagenesis may reveal the presence of a second, distinct active site. Met8p contains no sequence homology to other metal ion chelatases, and only weak homology, beyond the NAD-binding domain, to any other known proteins.

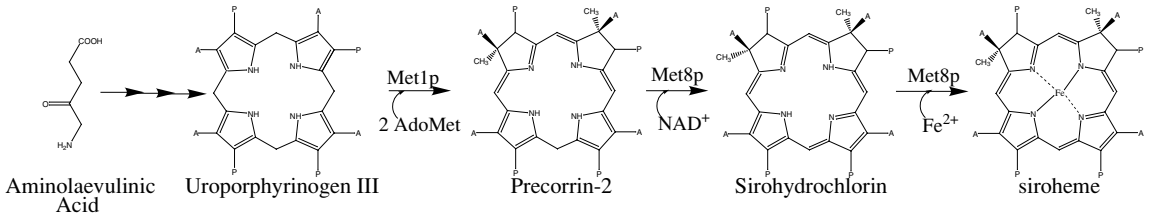

Figure 1. Siroheme biosynthesis in *Sacchromyces cervisiae*. Met8p is responsible for two enzymatic steps between the common tetrapyrrole intermediate, uro'gen III and siroheme.

SRUCTURE DETERMINATION

Crystals of Met8p were grown by the hanging drop crystallization method by mixing an equal volume of purified met8p (7 mg ml^{-1}) with a well solution containing 18-22 % polyethylene glycol (MW = 4000), 0.2 M Li_2SO_4, 0.1 M Tris, pH 8.5, and 3% methylpentanediol (MPD), 1 mM DTT. A complete native data set was collected at the EMBL-Hamburg Synchrotron on station BW7B (Table 1).

Selino-methionine substituted Met8p (containing a N-terminal His-tag) was prepared using the methionine inhibition method [2] and purified according to traditional Ni^{2+} chelating sepharose methods followed by gel filtration. The presence of SeMet was unambiguously confirmed by

amino acid analysis, where the wild-type protein exhibited a strong Met peak, and the Se-Met substituted protein gave no Met peak due to complete destruction of the SeMet during the analysis.

Multiple anomalous dispersion, MAD data was collected at the ALS, station 5.02. Three wavelengths were collected using a 30º slice, reverse beam protocol in the order of peak, remote and inflection point. Complete data was collected in three, 360º sweeps (Table 1). The data were processed and scaled using the HKL set of programs, setting the NOMERGE option [3].

Unmerged reflection files were input into SOLVE to identify the selenium substructure [4]. The program easily identified 12 sites over 10.7 σ (with a score of 76.24) and produced phases that were solvent flattened using RESOLVE [5]. The entire polypeptide chain was traced in this map and the model was refined though a series of model building and refinement cycles using the programs "O" and REFMAC [6,7].

Table 1. Data Collection Statistics

Title	Native	Se-peak	Se-remote	Se-inflect.
Wavelength (Å)	0.8445	0.97969	0.95372	0.97990
Resolution (Å)	20 - 2.1	20-2.7	20-2.7	20-2.7
Outer resolution shell (Å)	2.17-2.1	2.8-2.7	2.8-2.7	2.8-2.7
Observed reflections	1017319	492699	540295	496027
Unique reflections	64822	29993	32497	30123
% Completeness	99.0	98.5	98.3	98.3
Outer resolution shell	90.4	87.8	85.4	85.7
Rmerge^	0.045	0.084	0.068	0.069
Outer resolution shell	0.408	0.249	0.339	0.394
Mosaicity	0.523	0.793	0.756	0.742

^Rmerge = $\Sigma | I-\langle I\rangle | / \Sigma I$ where I is the intensity of an individual measurement and <I> is the average intensity from multiple observations.

STRUCTURE DESCRIPTION

Though the model is not yet complete a picture of the molecular dimer of met8p is beginning to emerge. Met8p forms a homodimer composed of two NAD-binding domains, one intertwined middle domain and two helical C-terminal domains. The entire shape resembles a large "X" with one polypeptide crossing the other (Figure 2). The N-terminal NAD-binding domain contains some of the familiar aspects of all nucleotide binding domains – namely the N-terminal GxGxxA/G sequence which manifests itself structurally as the C-terminal end of the domain's first β-strand.

The middle domain of the dimer is a tight mix of both polypeptides. The domain forms a triple layer structure, ββα, consisting two four stranded sheets packed in a β-sandwich laying over two helices. The N-terminus of each polypeptide of the dimer, residues 2-11, form the two internal β-strands, β1, of the first sheet and lie on the opposite side of the dimer two-fold from the NAD-binding domains that they precede. After forming the NAD-binding domain the polypeptide returns to complete the middle domain. The C-terminal domain lies on the opposite side of the molecular dimer from the NAD-binding domain of the same polypeptide. The C-terminal domain contains three long helices (and an extended loop) that lie parallel to the active site cleft above it.

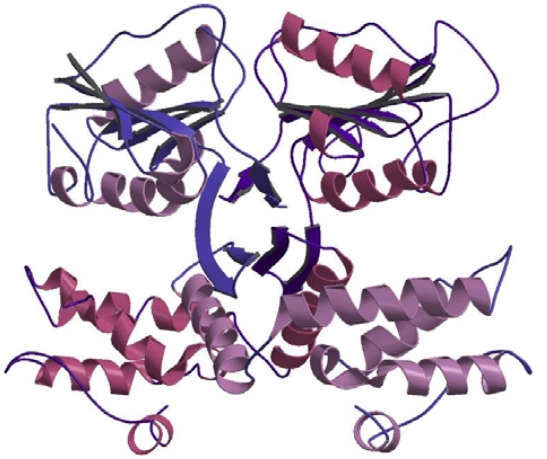

Figure 2. Structure of the Met8p homodimer, consisting of three intertwined domains.

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